Jl S. Afr. Bot. 44 (4): 381–386 (1978)

FLAVONOIDS OF THE SUMMER RAINFALL PROTEAS AND THEIR CHEMOTAXONOMIC SIGNIFICANCE

C. W. Glennie

(Department of Chemistry, University of the Witwatersrand, Johannesburg)

L. E. Davidson

(Department of Botany, University of the Witwatersrand, Johannesburg)

Abstract

Flavonoids were surveyed (by paper chromatography) in leaves of 15 summer rainfall *Protea* species. Five different 3–0–glycosides of quercetin were found, viz. the arabinoside, galactoside, glucoside, rhamnoside and rutinoside. Most species contained only 3 different glycosides but one species, *P. laetans* L. E. Davidson, contained all five. *P. laetans*, also, was the only species to contain leucoanthocyanins. All species, except three contained abutin and none contained cyanogenic compounds.

Uittreksel

FLAVONOÏEDE VAN DIE SOMERREËNSTREEK PROTEAS EN HULLE CHEMIES-TAKSONOMIESE BETEKENIS

'n Papierchromatografiese opname is gedoen van die voorkoms van flavonoïede in die blare van 15 *Protea* spesies van die somerreënstreek. Vyf verskillende 3–0–glikosiede van kwersetien is gevind, nl. die arabinosied, galaktosied, glukosied, rhamnosied, en rutinosied. Meeste spesies het slegs drie van die glikosiede bevat. *P. laetans* daarenteen al vyf. *P. laetans* was ook die enigste spesies wat leukoantosianiene bevat het. Al die spesies behalwe drie het arbutien bevat, maar geen sianogeniese stowwe nie.

Introduction

Comparatively little is known about the phytochemistry of indigenous South African plants. A study of the summer rainfall proteas was therefore carried out to determine their flavonoid glycoside content. This would be of value for several reasons. Mainly, it would serve to determine the chemical relationships between the various species in this group and also variations within species. A survey of flavonoids and other phenolic compounds has not yet been carried out and it would be worthwhile to screen for novel compounds.

Another reason for a chemical survey of these plants is the position of *Protea laetans*. This species was recently described by Davidson (1975) and it was desirable to examine its flavonoid content and compare this with related taxa. Also, *P. caffra*, *P. multibracteata*, and *P. rhodantha* all have very similar morphology and it would be of interest to know whether this similarity carried through to their flavonoid content.

Accepted for publication 19th May, 1978.

Flavonoids were chosen as the compounds of interest in this study because of their many desirable properties as summarised by Harborne (1967). Elsworth and Martin (1971) reported the possible presence of quercetin in four species reported here and also the possibility that kaempferol could be present in trace amounts. Since their work was done on acid-hydrolysed plant extracts it was decided to expand on this and attempt to isolate and identify all the unhydrolysed flavonoid glycosides for this study.

In another study, members of the Proteaceae were found to contain both hydroquinone and its glucosylated derivative arbutin. These compounds were reported to be present in all of the 29 species studied (van Oudtshoorn, 1963). To extend this survey, all plants in the present study were examined for the presence of arbutin. Recent work in this laboratory has established the presence of acylated forms of arbutin in several *Protea* spp. (Perold and Rosenberg).

METHODS

The plant material was collected from a variety of locations in South Africa and air dried. Voucher specimens are deposited in the Moss Herbarium, University of the Witwatersrand. The dried leaves were milled and extracted with 80% methanol. After concentration, the extract was applied to Whatman 3 MM paper; the flavonoids were separated and purified using n-butanol:acetic acid:water (4:1:5) (BAW), 15% acetic acid, and n-butanol:ethanol:water (4:1:2,2) (BEW) (Harborne, 1967). The flavonoid glycosides were compared with authentic samples by R_f values (Table 1) in BAW, phenol:water (500:125) (PhOH), 15% HOAc and water. The u v spectra of all five glycosides were similar, Band I absorbing at 258 nm and Band II absorbing at 362 nm in 80% methanol.

For comparison purposes two-dimensional paper chromatograms were run on Whatman No. 1 paper, using BAW and 15% HOAc. When viewed under uv light it was difficult to locate all the dark absorbing flavonoids amongst the blue fluorescent compounds. This was overcome by placing a fluorescent sheet of paper under the chromatogram which blended with the blue spots thus clearly showing the dark flavonoids.

All samples of plant material were examined for hydrogen cyanide-producing compounds in the following manner. A small sample of each aqueous plant extract was hydrolysed with β-glucosidase which was obtained commercially from sweet almond meat. The released HCN was detected by picric acid papers suspended over the solution in an enclosed round bottom flask. All positive tests were checked by the Dilute Hydrochloric Acid Test (Vogel, 1942).

The presence of leucoanthocyanins was determined by hydrolysing the plant extracts with 2N HCl in a test tube and then extracting with amyl alcohol. Delphinidin and cyanidin were identified by comparison with authentic standards in respect to R_f values on paper chromatograms and u v maxima (Harbone, 1973).

Unhydrolysed, methanolic plant extracts were used for the survey of arbutin and hydroquinone. Spots of these extracts were applied to Whatman No. 1 paper chromatograms which were developed overnight in BAW or BPW (n-butanol:pyridine:water-6:4:3). After air drying, the chromatograms were sprayed with Pauly's Reagent (0,5 % solution of diazotised sulfanilic acid in 10 % aqueous Na_2CO_3). The arbutin and hydroquinone appeared as red spots on a white or pale cream background.

TABLE 1.

R_f Values of Phenolic Glycosides of Summer Rainfall Protea Species.

Compound	R_f values (x100) in			
	BAW	PhOH	15 % HOAc	H_2O
Quercetin 3-arabinoside	70	61	33	07
Quercetin 3-galactoside	62	62	48	12
Quercetin 3-glucoside	58	62	35	06
Quercetin 3-rhamnoside	77	56	55	22
Quercetin 3-rutinoside	49	46	58	27
Arbutin	48	BPW 65		
Hydroquinone	84	87		

RESULTS AND DISCUSSION

The flavonoids in the leaves of twenty-six different samples of fifteen species of *Protea* are reported in Table 2. Along with the chemical results are also shown the geographical location from which the plant sample was collected. All plants contained only the one aglycone, quercetin, and any variations arose from the different 3-0-glycosides present. Flavonols are the flavonoids generally associated with the more primitive woody members of the Dicotyledoneae. The presence of the flavonol quercetin in this group of proteas suggests that it is rather primitive. Also, since it is the only aglycone present and has a simple glycosylation pattern, all members of this group are probably of a similar evolutionary age.

Quercetin has been reported previously in four of the species studied here (Elsworth & Martin, 1971; van Oudtshoorn, 1963). The presence of kaempferol was reported in two of these species but in this study no kaempferol could be found. The plants showed four different patterns of quercetin glycoside distribution. The galactoside, glucoside and rhamnoside were distributed throughout while the arabinoside and rutinoside were restricted in their distribution.

P. multibracteata and *P. rhodantha* showed chemical variation within the species while all other species had only one pattern of flavonoids. Four samples of *multibracteata* contained three of the quercetin glycosides while one sample contained an extra glycoside. *P. rhodantha* exhibited three different glycoside patterns. As can be seen from Table 2 *P. multibracteata* and *P. rhodantha* are the only two species examined which show the property of chemical variation.

It is probable that the rutin (quercetin-3-0-rhamnoglucoside) found in these plants is biosynthesized from quercetin-3-0-glucoside by the addition of rhamnose. This sequence of building up diglycosides of flavonoids from the monoglycosides has been suggested by Harborne (1975).

All plants in this study were tested for cyanogenesis and found negative. Hegnauer (1969) reported that *P. cynaroides* L., one of the winter rainfall *Protea* species, gave a positive test for HCN. This is the only member of the sub-family Proteoideae which was reported positive. (All the South African Proteaceae except for one genus, *Brabeium*, belong to this sub-family.) The present study yielded contrary results, for when material of *P. cynaroides* was collected from three different sources, it was found negative in all cases. On the other hand, ten of thirty-seven species of *Leucadendron* and *Leucospermum* tested for cyanogensis gave positive results (Glennie).

The only species which contained leucoanthocyanins was *P. laetans*. The anthocyanins released on acid hydrolysis of the plant material were identified as cyanidin and delphinidin. In addition to being the only species to contain leucoanthocyanins, *P. laetans* is the only species to contain all five quercetin glycosides. These two properties appear to be unique to *P. laetans* and delimit this new species from the others.

All plant samples of summer rainfall proteas, with the exception of *P. rubropilosa*, *P. nubigena* and *P. comptonii*, contained arbutin and many contained its aglycone hydroquinone. It is probable that the hydroquinone came from the breakdown of arbutin during the collecting and drying of the plant material. For this reason the distribution pattern of hydroquinone is not considered, as it could be of dubious value. Including this study, arbutin has now been reported present in 41 species of 6 genera in the Proteoideae. Outside this sub-family and the Ericaceae, where it is widespread, its occurrence is rare and erratic, being found mainly in *Malus* and *Pyrus* spp.

Arbutin was found to be absent from *P. comptonii* and *P. rubropilosa* and to be replaced by several esters of 2-hydroxy-4-hydroxymethylphenylalloside. Both 6-0-cinnamate and 6-0-benzoate of 2-hydroxy-4-hydroxymethylphenyl β-D-allopyranoside were found in these two species (Perold, Beylis and Howard, 1973b). The newly described species, *P. nubigena*, also does not contain arbutin but neither does it contain the same replacement compounds as *P. rubropilosa*

TABLE 2

Distribution of Phenol Glycosides in Summer Rainfall *Protea* Species, by paper chromatographic analysis.

Species	*Compound* 1	2	3	4	5	6	*Geographical Source*
P. caffra Meissner		+	+	+		+	Magaliesburg
P. comptonii Beard		+	+	+			Haenertsburg
P. curvata N.E.Br.		+	+	+	+	+	Barberton
P. dracomontana Beard		+	+	+		+	Drakensberg
P. gaguedi J. F. Gmelin		+	+	+		+	Barberton (2 samples)
P. laetans L. E. Davidson	+	+	+	+	+	+	Blyde River (2 samples)
P. multibracteata Phillips		+	+	+		+	Oribi, S. Coast Natal
P. multibracteata Phillips		+	+	+		+	Port St Johns (2 samples)
P. multibracteata Phillips		+	+	+		+	Kokstad
P. multibracteata Phillips	+	+	+	+		+	S. Coast Natal
P. nubigena Rourke		+	+	+			Tugela Gorge
P. parvula Beard		+	+	+	+	+	Dullstroom
P. rhodantha Hook f.		+	+	+		+	Nels Hoogte
P. rhodantha Hook f.		+	+	+		+	Blyde River
P. rhodantha Hook f.		+	+	+		+	Long Tom Pass
P. rhodantha Hook f.		+	+	+		+	Iron Crown
P. rhodantha Hook f.	+	+	+	+		+	Iron Crown
P. rhodantha Hook f.		+	+	+	+	+	Blyde River
P. rhodantha Hook f.		+	+	+	+	+	Haenertsburg
P. rhodantha Hook f.		+	+	+	+	+	Sheba (2 samples)
P. rubropilosa Beard		+	+	+			Blyde River
P. roupelliae (dwarf form) Meissner		+	+	+		+	Barberton
P. simplex Phillips		+	+	+		+	S. Coast Natal
P. transvalensis Phillips		+	+	+		+	Haenertsburg
P. transvalensis Phillips		+	+	+		+	Iron Crown
P. welwitschii Engel		+	+	+	+	+	Johannesburg

Key 1 – Quercetin-3-0-arabinoside, 2 – Quercetin-3-0-galactoside,
3 – Quercertin-3-0-glucoside, 4 – Quercetin-3-0-rhamnoside,
5 – Quercetin-3-0-rutinoside, 6 – Arbutin.

and *P. comptonii*. In *P. nubigena* arbutin appears to be replaced by several phenolic compounds which are yet to be identified.

It is of interest that *P. comptonii* and *P. rubropilosa* lack arbutin and that both contain the replacement glycosides, rubropilosin and pilorubrosin. They also contain the same complement of flavonoids, viz. the galactoside, glucoside and rhamnoside of quercetin. These species have similar morphology which tends to distinguish them from related taxa and one of the features separating them from each other is their geophysical distribution.

P. comptonii is found in a small area south of Barberton, Transvaal, while *P. rubropilosa* is found on the escarpment of the north-eastern Transvaal. Previously, the 6-0-benzoate of 2 hydroxy-4-hydroxymethylphenyl—D-gluco-pyranoside had been isolated from the winter rainfall *Protea lacticolor* Salisb. (Perold, Beylis and

Howard, 1973a). It appears that this ester also replaces arbutin in *P. lacticolor* as no arbutin or hydroquinone could be detected.

Two different samples of *P. gaguedi* were collected and examined. Both of the plants examined have similar morphology and inhabit the same ecological zone. The main difference between these samples is their time of blooming; one blooms in August while the other blooms in October. This time of flowering appears to be the only difference as both plants exhibited identical flavonoid and arbutin content as well as cyanogenic properties.

ACKNOWLEDGEMENT

Professor G. W. Perold (Department of Chemistry) is thanked for his encouragement and for his support of this work. Dr. J. P. Rourke (National Botanic Gardens of South Africa, Kirstenbosch) is thanked for supplying a sample of *Protea nubigena*.

REFERENCES

DAVIDSON, L. E., 1975. A New species of *Protea* in the Transvaal. *Jl S. Afr. Bot.* **41:** 207–210.

ELSWORTH, J. F. and MARTIN, K. R., 1971. Flavonoids of the Proteaceae, Part 1. A Chemical Contribution to Studies on the Evolutionary Relationships in the S. African Proteoideae. *Jl S. Afr. Bot.* **37:** 199–212.

GLENNIE, C. W. Unpublished results.

HARBORNE, J. B., 1967. *Comparative Biochemistry of the Flavonoids*. London and New York: Academic Press.

HARBORNE, J. B., 1973. *Phytochemical Methods*. London: Chapman and Hall.

HARBORNE, J. B., 1975. Flavone and Flavonol Glycosides. In: Harborne, J. B., Mabry, T. J. and Mabry, Helga (eds.), *The Flavonoids*. London: Chapman and Hall.

HEGNAUER, R., 1969. *Chemotaxonomie der Pflanzen*. Volume 5. Basel and Stuttgart: Birkhauser.

PEROLD, G. W., BEYLIS, P. and HOWARD, A. S., 1973a. *J. chem. Soc. Perkin* **I:** 1973, 638–643.

PEROLD, G. W., BEYLIS, P. and HOWARD, A. S., 1973b. *J. chem. Soc. Perkin* **I:** 1973, 643–649.

PEROLD, G. W. and ROSENBERG, M. E. K. Unpublished results.

VAN RHEEDE VAN OUDTSHOORN, M. C. B., 1963. Distribution of phenolic compounds in some South African Proteaceae. *Planta med.* **11:** 399–406.

VOGEL, A. I., 1942. *Qualitative Chemical Analysis*. London: Longmans, Green.